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Different turnover rate of hepatitis C virus clearance by different treatment regimen using interferon-beta

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Background/Aim: **Since patients with high viral load and HCV subtype 1b are known to respond poorly to interferon (IFN) therapy, the viral dynamics of HCV RNA after initiation of interferon therapy were examined in the present study with respect to two different administration regimens, once *vs.* twice a day.**
Methods: **Twenty-two patients with chronic hepatitis C confirmed by liver biopsy and with >1 Meq/ml of HCV RNA and HCV subtype 1b were randomly assigned to two different IFN administration regimens (6 million units of IFN once a day or 3 million units of IFN twice a day), and the serum HCV RNA level was serially measured.**
Results: **Graphs of HCV RNA levels *vs.* treatment time showed an initial rapid fall, followed by a slower clearance phase. Fitting the data to a model for HCV decay proposed by Neumann et al. showed that the treatment efficacy was significantly higher with twice daily administration. Negativity for HCV RNA measured by Amplicor assay in the twice-a-day administration group was 18%, 73% and >89% at 1, 2 and 3 weeks, respectively, in contrast to 0%, 0%, and 18%, respectively, with once-a-day administration. However, a significant reduction of platelet count and albumin level, a marked increase in serum aspartate aminotransferase/alanine aminotransferase, and a high incidence of renal toxicity (proteinuria) were found in patients receiving IFN twice a day in comparison with those receiving it once a day.**
Conclusion: **The twice-a-day administration of IFN accelerated the clearance of HCV RNA from serum, leading to a more efficient virological response for patients with chronic hepatitis C, but with a high rate of renal toxicity.**

Key words: **Amplicor-HCV monitor assay; HCV RNA level; Interferon-beta; Twice-a-day administration; Viral dynamics.**

PATIENTS with chronic hepatitis C exhibit persistent viremia and slowly progressive liver disease (1,2). Interferon (IFN) is effective for patients with chronic hepatitis C (3), but the efficacy of IFN depends on host and viral factors (4–10); IFN is effective in patients without liver cirrhosis, and with low viral load and HCV subtype 2a. However, patients with high viral load and hepatitis C virus (HCV) subtype 1b are known to respond poorly to IFN; the sustained virological response rate was only 4–7% among these patients with 48 weeks of IFN monotherapy (11,12).

The pretreatment viral load is an independent factor related to the IFN response. The best indicator of a favorable outcome of IFN treatment is the initial alanine aminotransferase (ALT) and HCV RNA response. Recent studies showed that the change in serum HCV RNA during the early weeks of therapy, especially the loss of HCV RNA by week 4 after initiation of IFN therapy, is one of the predictors of sustained response (13–15). In one study (13), the rate of decline observed within 14 days after treatment was predictive of viral negativity at 12 weeks. Monitoring the rate of

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decline in HCV level may allow confident identification of patients who will or will not respond to further treatment (16), and may be used to signal a potential need for treatment modification.

Despite the obvious importance of viral replication in patients with chronic hepatitis C, until recently relatively little information has been available regarding the kinetics of virus production and clearance *in vivo*. Studies in human immunodeficiency virus (HIV)-1 infected patients showed a rapid half-life for HIV-1, indicative of a dynamic process involving continuous rounds of *de novo* virus infection, replication and rapid cell turnover (17–19). These findings led to the examination of whether other viruses may also replicate continuously and be highly productive *in vivo*. Recently, dynamics of HCV after IFN administration have been investigated in patients with chronic hepatitis C (20–23), and yielded estimates of drug effectiveness, the HCV clearance rate, and the rate of infected cell loss (22).

Antibacterial drugs are usually administered every 6 to 8 h to maintain an effective concentration above the minimum inhibitory concentration (MIC) (24,25). IFN affects intracellular signalling pathways, and inhibits viral proliferation (26,27). An increased activity of 2′–5′ oligoadenylate synthetase (2′–5′ OAS) induced by IFN is effective for inhibition of replication of some virus in a cell. Interferon has a half-life of 5–7 h in serum (28), and thus increasing the frequency of drug administration might increase its antiviral effect.

IFN-beta is effective for the treatment of patients with HCV infection (29,30). Since patients with high viral load and HCV subtype 1b are known to respond poorly to IFN therapy given daily or 3 times a week, the present study was conducted to examine the effects of more frequent administration. Here, regimens of IFN-beta, given once or twice a day, for the clearance of HCV in patients with high viral load and HCV subtype 1b were compared by closely monitoring the virus decay with IFN. The model of Neumann et al. (22) was used to analyze the data, and estimate the effectiveness of two different IFN administration regimens.

Materials and Methods

Patients with chronic hepatitis C

The design of this study was discussed and established in May, 1996. Patients with chronic hepatitis C, and positivity for HCV RNA antibody confirmed by anti-HCV ELISA assay (Ortho) or PHA test (Dinabott), were examined to determine serum HCV RNA quantitation and HCV subtype. HCV RNA level in serum was measured by branched DNA (bDNA) probe assay (Chiron, Daiichi, Tokyo, Japan), and HCV subtypes by the method of Okamoto et al. (31). These patients underwent liver biopsy, and the fibrosis stage of the liver was assessed according to Desmet et al. (32).

TABLE 1

Demographic features of patients receiving an intravenous injection of interferon (IFN)-beta once or twice a day

	Once daily	Twice a day	*p*-value
No. of patients	11	11	
Mean age (range)	50±9.4 (37–65)	44±14 (25–63)	0.330
Sex (male:female)	11:0	6:5	0.035
History of blood transfusion	3/11	3/10	1.000
Albumin (g/dl)	4.0±0.3	4.1±0.3	0.422*
AST (IU/l)	52±26	63±29	0.353*
ALT (IU/l)	91±59	104±56	0.609*
Total Bil	0.68±0.26	0.67±0.21	0.874*
AL-P	181±61	172±56	0.732*
r-GTP	47±22	40±18	0.426*
T Chol	178±44	161±36	0.336*
BUN	14±4	14±4	0.851*
Cr	0.8±0.2	0.7±0.2	0.435*
RBC (10^4/mm^3)	470±51	466±41	0.848*
WBC (/mm^3)	6100±1900	5400±1200	0.355*
Platelet count (10^3/mm^3)	200±47	166±45	0.100*
Urine protein	0/11	0/10	1.000
Urine sugar	0/11	0/10	1.000
Occult blood in urine	0/11	0/10	1.000
Viral load			
Amplicor HCV monitor (Kcopies/ml)	485±249	422±244	0.555*
bDNA probe assay (Meq/ml)	7.9±5.7	6.7±5.7	0.636*
HCV subtype 1b	11	11	1.000

p-value: Fisher's exact test and *Welch's test.

AST: aspartate aminotransferase; ALT: alanine aminotransferase; Total Bil: total bilirubin; AL-P; alkaline phosphatase; r-GTP: γ-glutamyl transpeptidase; T Chol: total cholesterol; BUN: blood urea nitrogen; Cr: creatinine; RBC: red blood cell count; WBC: white blood cell count; HCV: hepatitis C virus.

Patients with HCV RNA load of >1 Meq/ml, HCV subtype 1b, and fibrosis stage from F1 to F3 were enrolled in this study. Patients with liver cirrhosis (F4 stage by the criteria of Desmet et al.), autoimmune hepatitis, alcoholic liver injury, or positivity for HBs antigen were excluded from this study. In addition, patients with white blood cell counts of<3000/mm^3 or platelet counts of<70 000/mm^3 were excluded. After obtaining informed consent from each patient, the viral, biochemical and histological data of each patient were submitted to the Supervisory Committee set up at the Registration Office to check eligibility for enrollment in this study.

This study was approved by the ethical committee of each institute in accordance with the Helsinki Declaration. Patients were enrolled during September, 1996 and August, 1997.

IFN treatment regimens

The eligible 23 patients were randomly assigned to two different IFN regimens: 6 million units (MU) of interferon (IFN)-beta once a day, or 3 MU of IFN-beta twice a day for 28 days. One patient did not receive IFN-beta, at his request, and the remaining 22 patients were enrolled and defined as a full analysis set.

Once-a-day administration: Eleven patients received an intravenous (iv) injection of 6 MU of IFN-beta (Toray Industries Inc., Tokyo, Japan) once a day. In brief, 6 MU of IFN-beta was dissolved into 100 ml of physiological saline solution. IFN was administered by a 30-min drip infusion at 9:00 every morning.

Twice-a-day administration: Eleven patients received an iv injection of 3 MU of IFN-beta twice a day. In brief, 3 MU of IFN-beta was

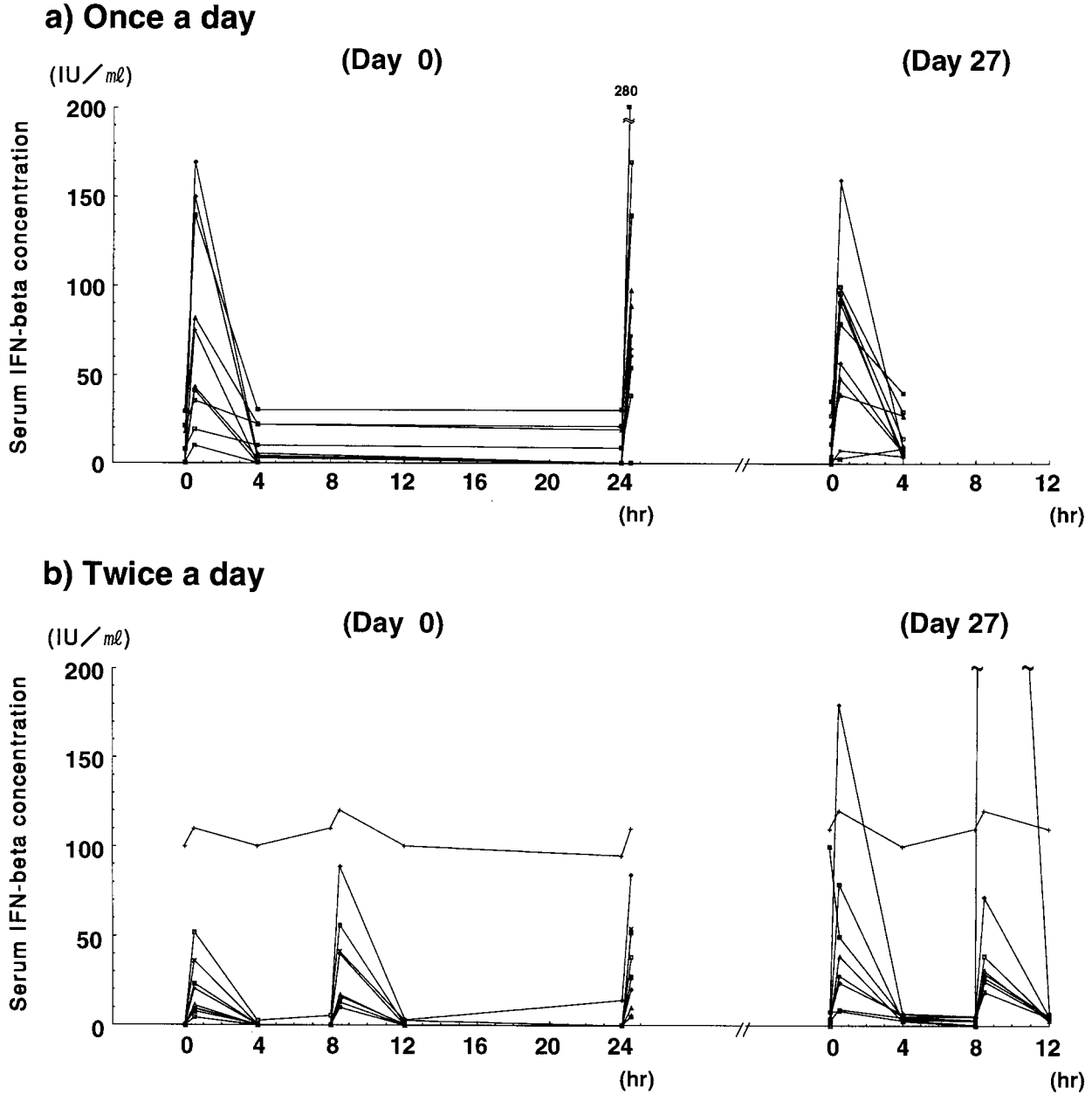

Fig. 1. Serum interferon (IFN) concentration. Serum concentration of IFN was measured before and at the completion of IFN infusion on day 0 and 27.

dissolved in 100 ml of physiological saline solution. IFN was administered by a 30-min drip infusion twice a day at 9:00 and 17:00.

Sampling of blood

Blood was collected from the patients on the following schedule: on Day 0 and 27, blood was collected at 9:00 (before iv injection of IFN), at 9:30 (at the completion of IFN infusion), 13:00, 17:00, and 21:00. On Day 1, blood was collected also at 9:00 and 9:30. On Day 2, 3, 7, 10, 14, 17, 21 and Day 28, blood was collected at 9.00. In the patients receiving IFN twice a day, blood was further collected at 17:30 (at the completion of the 2nd infusion of IFN) on Day 0 and 27.

Blood was centrifuged at 450 × *g* for 15 min, and stored in sterile stock tubes at −20°C until used.

Serum IFN concentration and 2′–5′ OAS activity

IFN concentration in serum was measured by ELISA (Toray-Fuji Bionics, Tokyo, Japan), and 2′–5′ oligoadenylate synthetase (2′–5′ OAS) activity by a 2–5A kit "EIKEN" (Eiken Chem., Tokyo, Japan) using stored blood samples collected and stored as described above.

Quantitation of HCV RNA

HCV RNA level in serum was measured by the Amplicor-HCV monitor assay (Roche Molecular Diag., Tokyo, Japan) using blood samples collected at 9:00, 13:00, 17:00, and 21:00 on Day 0, and at 9:00 on Day 1, 2, 3, 7, 14, 21, and 28. Since serum HCV RNA levels of <2000 copies/ml were not detected by Amplicor-HCV monitor assay, they were further examined by Amplicor quality assay (detection limit= 200 copies/ml). Negativity of HCV RNA by the latter assay was set as HCV RNA negative in this study.

Biochemical parameters

Biochemical parameters of blood (aspartate aminotransferase (AST), ALT, total protein, albumin, r-GTP, Al-P, total bilirubin, blood urea nitrogen (BUN), creatinine (Cr), total cholesterol, amylase), blood cell count (RBC, Ht, Hb, white blood cell count, neutrophils, platelet count), and biochemical parameters of urine (protein, sugar, occult blood) were measured on Day 0, 2, 3, 7, 10, 14, 17, 21 and 28.

Model of HCV RNA clearance

The model and technique presented by Neumann et al. (22) was used to analyze data for each patient with the original equations and with modified equations; the sequential HCV RNA values measured by Amplicor-HCV monitor assay. The clearance curve of HCV RNA was divided into the initial rapid clearance phase (≤2 days) followed by a slow clearance phase (>2 days).

Initially, the HCV RNA level at the initial clearance phase was calculated by the following model proposed by Neumann et al. (22):

$$V(t) = V_0\{1-E+E\exp[-C(t-t0)]\}$$

where t is the time since treatment was initiated, and V(t) is viral level at time t. The parameter V_0 is the viral load prior to IFN administration; E, the efficacy of treatment; C, the clearance rate of virus; and t_0, the pharmacological delay.

Using the values for V_0, E, C, and t_0 obtained from data between days 0 and 2, we then fit the entire data set (days 0–28) (using a nonlinear least squares regression technique) to the equation:

$$V(t) = V_0\{A\exp[-L_1(t-t_0)]+(1-A)\exp[-L_2(t-t_0)]\}$$

where

$$L_{1,2} = 1/2\times\{(C+D)\pm\sqrt{(C-D)^2+4(1-E)\,CD}\}$$

$$A = \frac{EC-L_2}{L_1-L_2}$$

Here D, the death rate of infected cells, was allowed to vary.

However, for some of the patients the viremia rapidly fell to undetectable levels, limiting the ability to use the full solution published

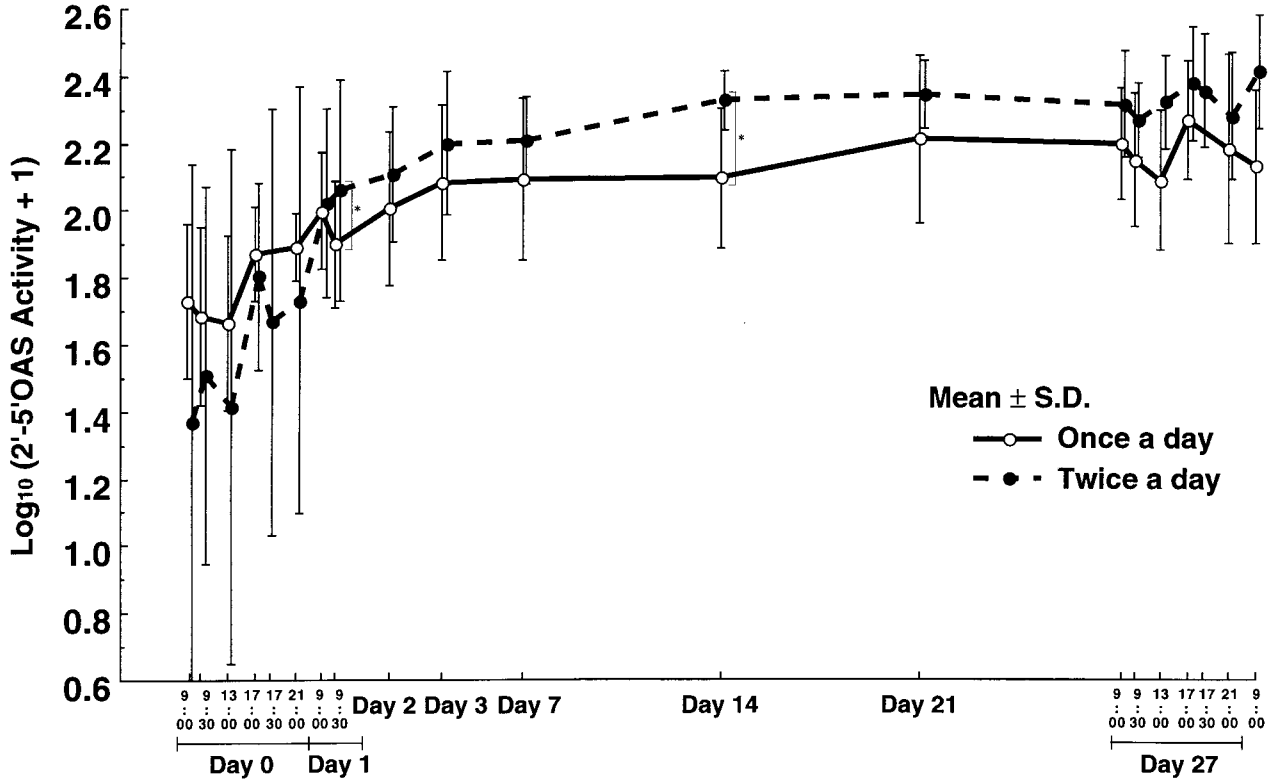

Fig. 2. Serum 2′–5′ oligoadenylate synthetase (2′–5′ OAS) activity. 2′–5′ OAS activities in serum 2 days after initiation of interferon (IFN) therapy with the twice-a-day administration of IFN were similar to those with once-a-day administration.

by Neumann et al. (22). Thus, we fit the available data to the following modified equations, based on the solutions in Neumann et al. (22); we assumed that death of infected cells could be ignored during the first 2 days of therapy, but was included for later times:

$$V(t)=\begin{cases} V_0 & \text{if } t < t_0 \\ V_0\{1-E+\exp[-C(t-t_0]\} & \text{if } t_0 \le t \le 2 \text{ days} \\ V_0\{A\exp[-L_1(t-t_0)]+(1-A)\exp[-L_2(t-t_0)\} & \text{if } t > 2 \text{ days} \\ t_0 = 8 \text{ h} \end{cases}$$

Statistical analysis

Statistical analysis was performed using Fisher's exact test, the Wilcoxon two-sample test, and the *t*-test. HCV RNA negativity rate was analyzed by the Kaplan Meier method with log-rank test. A *p*-value <0.05 was considered statistically significant.

Results

Profiles of patients

The demographic profiles of the patients in the two groups (once- and twice-a-day administration) were similar except for sex (Table 1). Virological features of HCV RNA levels were also similar.

Tolerance: During administration of IFN twice a day, IFN was discontinued in two patients at Day 14 due to severe adverse events of liver dysfunction (serum ALT/AST levels >700 IU/l) and/or severe proteinuria (urinary protein excretion >4 g/dl, and serum albumin level <3.0 g/dl).

IFN concentration in serum

IFN concentration in serum was measured on Day 0 and 27 (Fig. 1). On Day 0, peak serum IFN concentration reached the level of 83±59 IU/ml at the end of the 30-min injection of IFN in patients receiving the IFN once-a-day treatment regimen, in contrast to levels of 26±31 and 39±37 IU/ml among patients receiving IFN twice a day. On Day 27, the peak levels of IFN were similar to the previous values shown on Day 0 in patients receiving IFN once a day, whereas it increased to 60±58 IU/ml in patients receiving IFN twice a day. Thus, the level of IFN increased by Day 27 in patients receiving IFN twice daily, and it appears it

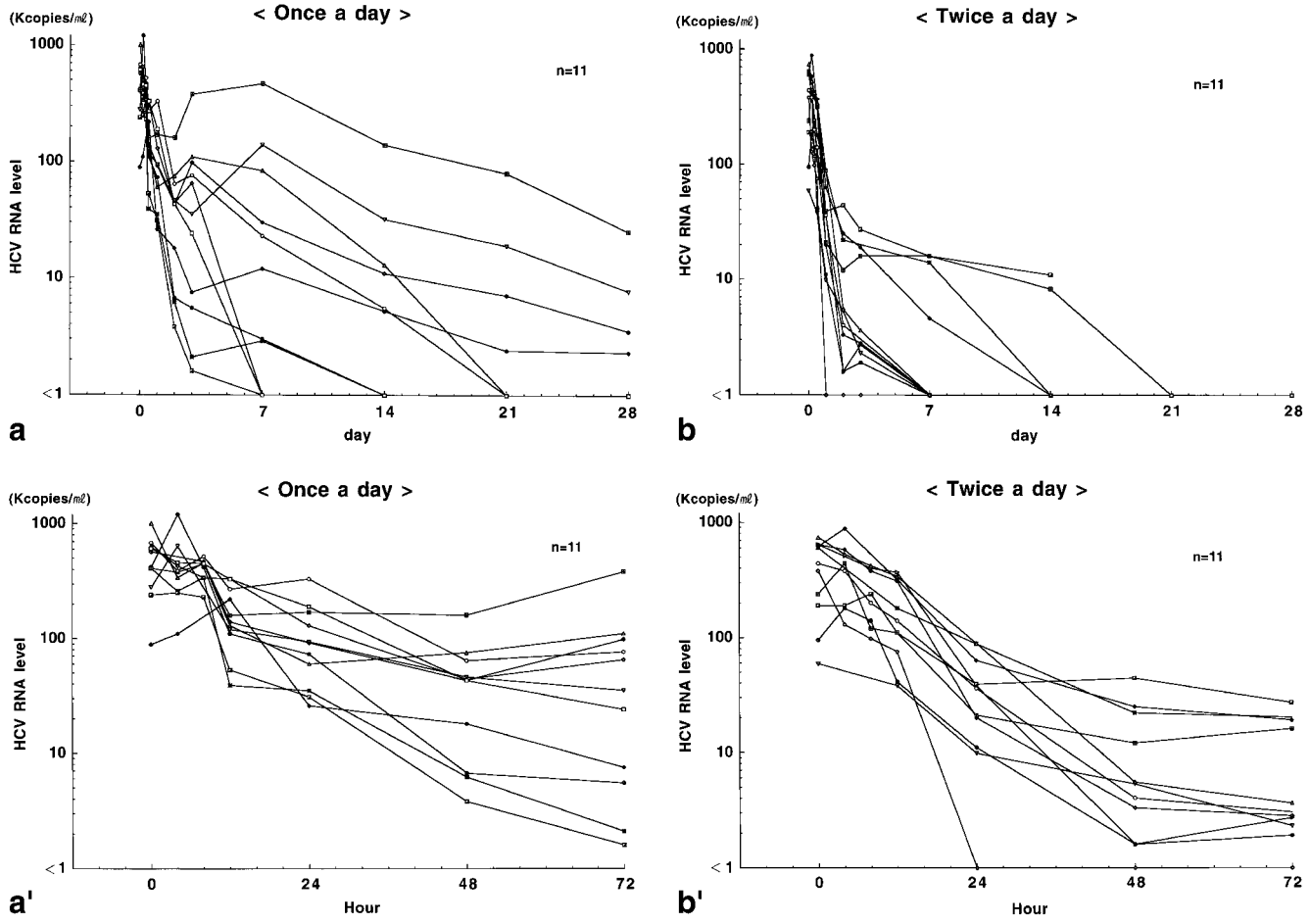

Fig. 3. Scattergram of serum HCV RNA levels. HCV RNA level in serum was serially measured by Amplicor-HCV monitor assay. (a) and (a') Once a day administration of interferon (IFN)-beta (6 MU × 1 per day). (a; Day 0 to Day 28, a'; during initial 72 h). (b) and (b') Twice a day administration of IFN-beta (3 MU × 2 per day). (b; Day 0 to Day 28, b'; during initial 72 h).

TABLE 2

Fitting model for the clearance of HCV RNA

Patient	V_0 (kcopies/ml)	t_0 (h)	C (1/day)	E (%)	D (1/day)	CV_0
Once daily IFN treatment (6MU × 1)						
1	530	8	1.7	97.5	0.13	904
4	574	8	2.8	93.2	0.16	1581
5	527	8	1.5	89.4	0.19	775
7	1000	8	16.2	93.3	0.16	16150
10	410	8	165.6	60.2	0.06	67896
11	153	8	2.9	89.9	0.08	441
13	280	8	2.3	99.9	0.00	630
15	226	8	2.4	80.3	0.05	539
17	154	8	2.8	98.4	0.04	430
19	566	8	11.1	89.0	0.12	6264
22	410	8	16.7	96.4	0.13	6832
Mean	439	8.0	20.5	89.9	0.10	9313
SD	245	0.0	48.5	11.3	0.06	20019
Twice daily IFN treatment (3 MU × 2)						
2	417	8	11.1	99.8	0.00	4630
3	350	8	3.7	99.1	0.06	1291
6	662	8	3.1	99.7	0.03	2050
8	237	8	4.9	94.9	0.13	1151
9	616	8	4.1	96.0	0.15	2527
12	84	8	3.3	98.5	0.01	275
14	62	8	3.6	91.7	0.09	224
16	198	8	5.5	79.7	0.11	1097
18	694	8	5.5	99.5	0.05	3827
20	442	8	3.0	95.5	0.14	1334
23	690	8	4.3	99.8	0.00	3000
Mean	405	8.0	4.7	95.9	0.07	1946
SD	239	0.0	2.3	6.0	0.06	1418

The HCV RNA level at the initial clearance phase was calculated by the following model:

$$V(t) = V_0\{1-E+E\exp[-C(t-t_0)]\}$$

where t is the time since treatment was initiated, and V(t) is viral level at time t. The parameter V_0 is the viral load prior to IFN administration (baseline viremia); E, the efficacy of treatment; C, the clearance rate of virus; and t_0, the pharmacological delay. Using the values for V_0, E, C, and t_0 obtained from data between days 0 and 2, we then fit the entire data set (days 0–28) (using a nonlinear least squares regression technique) by the equation

$$V(t) = V_0\{A\exp[-L_1(t-t_0)]+(1-A)\exp[-L_2(t-t_0)]\}$$

where

$$L_{1,2} = 1/2\times\{(C+D)\pm\sqrt{(C-D)^2+4(1-E)CD}\}$$

$$A = \frac{EC-L_2}{L_1-L_2}$$

D, the death rate of infected cells. For baseline viral load to be relatively constant before treatment, the extracellular viral production rate must equal the viral clearance rate (C). Thus the production rate could be estimated as the product CV_0 times a factor equal to the extracellular fluid volume.

Parameter values for patient 5 were estimated without data set for days 0–2 *t*-test for E with outliers removed: two-tailed $p=0.03$ between the two groups.

also became nearly the same as patients receiving it once daily.

2′–5′ OAS activity

2′–5′ OAS activity in serum was measured every 4–12-h on Day 0 and 27, and at 9:00 on Day 1, 2, 3, 7, 14, and Day 21, indicating that the 2′–5′ OAS activity in serum of the patients receiving IFN twice a day was similar to that in patients receiving IFN once a day (Fig. 2).

Clearance of HCV RNA in serum

HCV RNA level in serum was measured by Amplicor-HCV monitor assay, and a graph of serum HCV RNA levels *versus* treatment time in 22 patients with chronic hepatitis C is shown in Fig. 3.

Fitting data to a model of HCV RNA clearance: The HCV RNA values measured by Amplicor-HCV monitor assay (Fig. 3, Table 2) were fit using nonlinear regression techniques to the model of Neumann et al. (22). Table 2 shows, for each patient, the parameter

TABLE 3

Fitting model for the clearance of HCV RNA with the modified equation

Patient	V_0 (kcopies/ml)	t_0 (h)	C (1/day)	E (%)	D (1/day)
Once daily IFN treatment (6MU × 1)					
1	469	8	1.4	96.1	0.63
4	404	8	1.0	99.4	0.00
5	488	8	1.6	85.2	0.26
7	537	8	18.5	77.8	0.26
11	133	8	2.4	90.8	0.07
13	483	8	3.5	98.6	0.14
15	423	8	12.4	81.7	0.09
17	195	8	3.1	98.8	0.15
19	516	8	11.1	86.1	0.13
22	240	8	3.5	98.3	0.11
Mean	389	8.0	5.8	91.3	0.18
SD	145	0.0	6.0	8.1	0.16
Twice daily IFN treatment (3 MU × 2)					
2	186	8	7.7	100.0	–
3	306	8	3.4	99.0	–
6	571	8	3.2	99.2	0.24
8	237	8	6.0	91.5	0.13
9	715	8	4.5	96.2	0.26
12	121	8	4.1	98.5	0.08
14	61	8	3.5	92.5	0.28
18	556	8	5.1	99.4	0.19
20	439	8	2.4	99.0	0.07
23	564	8	4.3	99.7	0.08
Mean	376	8.0	4.4	97.5	0.17
SD	223	0.0	1.5	3.1	0.09

The HCV RNA level was calculated by the following modified model:

$$V(t) = \begin{cases} V_0 & \text{if } t < t_0 \\ V_0\{1-E+\exp[-C(t-t_0]\} & \text{if } t_0 \leq t \leq 2 \text{ days} \\ V_0\{A\exp[-L_1(t-t_0)]+(1-A)\exp[-L_2(t-t_0)\} & \text{if } t > 2 \text{ days} \end{cases}$$

$$t_0 = 8 \text{ h}$$

Where the parameter V_0, the viral load prior to IFN administration (baseline viremia), E, the efficacy of treatment, C, the clearance rate of virus, t_0, the pharmacological delay (using a nonlinear least squares regression technique).
where

$$L_{1,2} = 1/2\times\{(C+D)\pm\sqrt{(C-D)^2+4(1-E)\,CD}\}$$

$$A = \frac{E\,C-L_2}{L_1-L_2}$$

D, the death rate of infected cells.
Parameter values for patient 5 were estimated without data set for days 0–2 ($p<0.05$ between the two groups by the Wilcoxon two-sample test).

values that allow the original model of Neumann et al. (22) to best fit the viral load data. The t_0, pharmacological delay, was assumed to be 8 h for all patients, and the outlying parameter was found in two cases: patients 10 and 16. When analysis was performed with all patients included, we were unable to find difference, two-tailed $p<0.05$, between the two groups in any of the parameters. However, excluding outlying parameter values, we were able to show that the efficacy of treatment (E) was significantly higher in the twice-a-day treatment group ($p=0.03$).

We then calculated the parameters using the modified equations, and the parameter estimates for each patient are presented in Table 3. Using the modified equations, treatment efficacy, E, was the only parameter found to be significantly different between the two regimens ($p<0.05$, the Wilcoxon two-sample test).

HCV RNA negativity in relation to the treatment regimens

HCV RNA level in serum was measured by Amplicormonitor assay. In all patients receiving IFN once a day, HCV RNA in serum could be detected for 2 weeks, and negativity of HCV RNA was demonstrated in 18% of the patients at 3 weeks and 36% at 4 weeks. In contrast, when patients received IFN twice a day, HCV RNA could be measured until Day 3 in all 11 subjects, but decreased to less than the detectable level in 18% at 1 week, 73% at 2 weeks, and 89% at 3 weeks, and in all subjects at 4 weeks. The HCV RNA negativity rate with twice-a-day administration was significantly higher, in comparison with that obtained with once-a-day administration, as analyzed using the Kaplan-Meier lifetable with log-rank test ($p=0.0002$).

Changes in biochemical parameters in relation to treatment regimens

The biochemical parameters, ALT and AST, increased in patients receiving IFN-beta twice a day, in contrast to a decrease in patients receiving IFN-beta once a day (Fig. 4). Albumin level in serum decreased in patients receiving IFN-beta twice a day. In addition, platelet counts were markedly reduced (to the level of 67 000/mm^3) in patients receiving IFN-beta twice a day, in comparison with those receiving IFN once a day.

Urine protein was detected in 73% (8 out of 11) and 100% of patients receiving IFN-beta once and twice a day, respectively (Table 4). Severity of proteinuria was significantly higher in patients receiving IFN-beta twice a day in comparison with those receiving IFN-beta once a day ($p=0.005$).

TABLE 4

Adverse effect of proteinuria

Treatment regimen	Severity of proteinuria (mg/dl)				*p*-value
	− (<25)	+ (25–65)	++ (65–200)	+++ (>200)	
Once a day ($n=11$)	3	4	3	1	
Twice a day ($n=10$)	0	1	3	6	0.005

Data were missing in one case in the twice-a-day regimen group.
p-value: the Wilcoxon rank sum test.

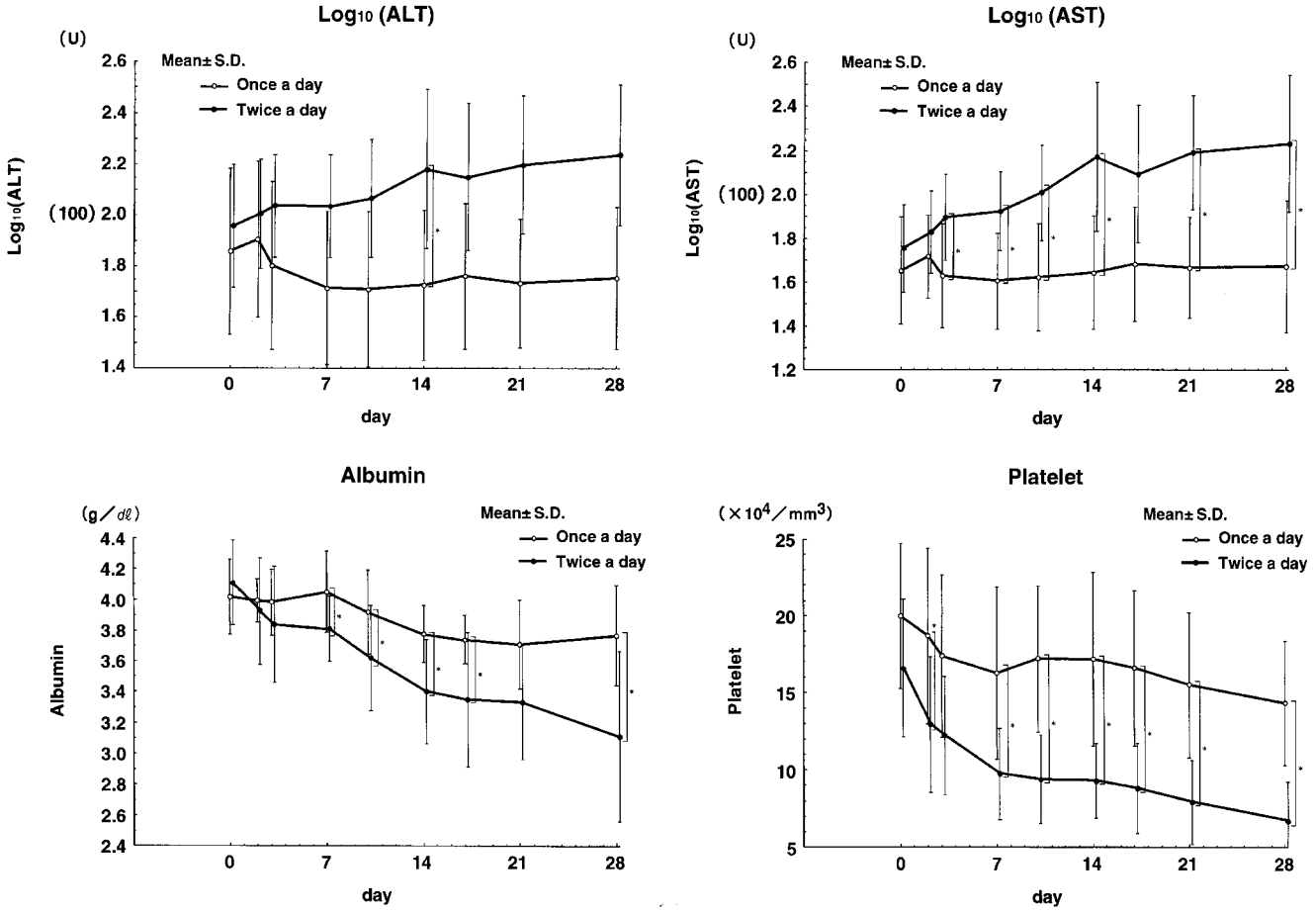

*Fig. 4. Changes in blood parameters during administration of interferon (IFN) once or twice a day. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, and platelet count were measured serially. Logarithmic transformation was performed on the variables of ALT and AST. The Wilcoxon test was performed using values that were subtracted from the values before treatment (*p<0.05).*

Discussion

In the present study, we investigated the dynamics of viral clearance by IFN-beta using two administration regimens: 6 MU once a day *vs.* 3 MU twice a day. Following the intravenous injection of 3 MU of IFN, peak serum concentrations of IFN reached a level that was half the level measured following the injection of 6 MU of IFN. However, 2′–5′ OAS activities in patients receiving IFN twice a day were similar to those in the once-a-day administration group. In addition, the decline of HCV RNA level was faster in the twice-a-day group.

The detection limit of HCV RNA was 0.5 Meq/ml with the bDNA probe assay (33), 2000 copies/ml with the Amplicor-HCV monitor assay, and 200 copies/ml with the Amplicor qualitative assay (34–36). Since the sensitivity of the bDNA assay method was poor, the HCV RNA level was below the detectable level in 36% of patients at 12 h; at 24 h, it was 46% in the once-a-day administration group, and 100% in the twice-a-day group (unpublished observation). On the other hand, since the sensitivity of the Amplicor method is superior to that of the bDNA probe assay, as of Day 15 HCV RNA negativity was not achieved among patients receiving IFN once a day, whereas it was demonstrated in 73% of patients in the twice-a-day treatment group. The HCV RNA negativity rate at week 4, measured by Amplicor assay, reached a level of 100% in the twice-a-day group, in contrast to 36% in patients who received a single daily administration of IFN, although the total dose of IFN was the same.

By analyzing the serum HCV RNA levels after IFN therapy, the rate of viral elimination from serum after initiation of antiviral therapy was determined. The clearance of HCV consists of at least two processes: the clearance of HCV RNA *per se*, and the elimination/suppression of virus-producing cells. In this study, the approach was to restrict the comparison to patients

who responded well to IFN. We noted that patient 10 (in the once-a-day group) had less than a half-log drop in serum by Day 2 and a viral load higher than baseline at 1 week, and that patient 16 (in the twice-a-day group) was the only patient who had less than a 1 log drop in serum HCV by Day 2 and had less than a 5-fold decline. These two patients were excluded from the comparison analysis.

Using the original and modified equation model of Neumann et al. (22), we were able to estimate the parameters describing the decline of HCV RNA following initiation of IFN-beta therapy. The clearance of HCV RNA was divided into two phases: an initial rapid clearance phase ($\leq$2 days), followed by a slower clearance phase. Excluding two patients who responded poorly to therapy, we were able to show that the efficacy of treatment (E) was significantly higher in the twice-a-day treatment group than the once-a-day group, with the original equation ($p=0.03$), as well as with the modified equations ($p<0.05$).

In this study, there was a significant sex difference between the once-daily group and the twice-a-day group, although the patients were randomly divided into two treatment groups at the Supervisory Committee. However, the decline of serum HCV in females in a twice-a-day group was similar to that in men (data not shown). Furthermore, HCV RNA measured by the Amplicor monitor assay is not linear above 500 kcopies/ml, and the high HCV RNA level could be underestimated by 10–20% in comparison with the value measured using diluted samples (36). Thus, the efficacy rate shown in this study may be underestimated.

A variety of adverse events were caused by the twice-a-day administration regimen: i.e., liver damage (increased serum ALT/AST concentration), decreased serum albumin, marked thrombocytopenia, and severe proteinuria. Thus, although early disappearance of HCV can be achieved by administering IFN twice a day, apparent renal toxicity was found in patients receiving IFN twice daily.

Hepatocyte damage and turnover can be estimated by surrogate parameters of aminotransferases which are released from hepatocytes, since AST and ALT are cytosolic enzymes mainly present in the liver. Though the serum ALT level declined towards the baseline value (normalized) with once-a-day administration of IFN, it increased in patients receiving IFN twice a day. However, this effect may play a minor role in the rapid clearance of HCV RNA, since hepatocyte damage was found several days later, after initiation of IFN therapy. As the vast majority of circulating plasma virus derives from continuous rounds of *de novo* virus infection, replication and cell turnover, the proposed *in vivo* antiviral mechanism of IFN may be more clearly evaluated when a reliable replication system in cell culture is available.

The kinetic data of HCV turnover shown in the present study and previous studies (22) suggest that protocols of IFN therapy should be modified to closely monitor the first few days after drug initiation, since the clearance rate from serum is faster during this period, and estimates of the efficacy of IFN treatment can be made. Prediction of end-of-treatment response and sustained response may also be defined by an early treatment response, and loss of HCV RNA during the initial weeks of IFN treatment may provide accurate identification of responders. Since our recent study showed that a high sustained response rate (75%) was achieved in patients receiving an additional 40 weeks of IFN after negativity for HCV RNA during initial treatment (37), there is a possibility that patients with early negativity of HCV RNA with a twice-a-day treatment may show a high sustained response rate with a subsequent 40-week treatment of IFN therapy including IFN-alpha 3 times weekly.

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